

FREEZING AND DRYING IN INTERTIDAL ALGAE ¹

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The intertidal region is an environment characterized by widely fluctuating conditions. It will be shown in this paper that in high latitudes the algae in this zone are exposed to extensive freezing and drying. These two aspects of immersion are considered together, since they have the common feature of cellular dehydration. In particular, their separate effects on the metabolism of algae have been investigated.

First will be discussed the effect of low temperatures in freezing a large amount of water in certain algae. Next, the natural dehydration that is caused by evaporation in several of the same species will be described. Finally, measurements will be reported which show a greatly depressed respiration in both the frozen and dried states. Some observations on the winter survival of *Fucus* in the Arctic are also included.

I. FREEZING

In the Woods Hole region in winter there are a number of macroscopic brown, green, and red algae exposed to freezing temperatures by the tide. They frequently feel brittle to a degree which suggests ice in them. Some of them have been observed imbedded in the ice at temperatures as low as -20° C. Conditions are even more rigorous in the Arctic where *Fucus* is a prominent intertidal alga. It may spend six months or more frozen in the ice at temperatures which go below -40° C. Since these plants contain 70 to 80 per cent water, it seemed pertinent to determine how much of this water, if any, is frozen at these extreme temperatures. Bieble (1939) reported that several intertidal algae would survive being frozen, but he made no quantitative determinations of the ice.

1. Method

Water gives off heat and also expands when it changes to ice. The expansion has been measured directly in a dilatometer by Moran (1935), Gortner (1937), and others to determine the amount of ice. Scholander *et al.* (1955) devised a flotation method to measure specific gravity and found as much as 90 per cent frozen water in the lichen *Cetraria richardsonii*. Volumetric methods were unsuitable here because of the difficulty of the dissolved gases that come out of solution on freezing. This would cause a density change which could not be separated from the same effect due to ice formation. Calorimetric determinations of ice, based on the conveniently large heat of fusion of water, have been used by Great-house (1935), Ditman *et al.* (1942), and others. Scholander *et al.* (1953) have recently reviewed these two methods.

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There has been no previous quantitative work on ice in algae. This author has described a simplified calorimetry for small animals (Kanwisher, 1955) which has been used here on several of the large intertidal algae. The calorimeter vessel is an ordinary Thermos bottle. The temperature of the water inside is read to 0.01° C. by a mercury thermometer through the stopper. The sensitivity is varied by changing the amount of water in the Thermos. Weighed pieces of ice are used for calibration.

A piece of the alga to be frozen, usually a few grams, is sponged free of excess water with filter paper, weighed, and placed in a small vial in a cold chamber set to the desired temperature. At least four hours is allowed for phase equilibrium between the water and ice in the alga. A check weighing at this point usually showed less than 1 per cent loss of water by evaporation.

The Thermos is thermally equilibrated with the desired amount of water and the temperature noted. Then the vial is removed from the cold chamber and alga immediately shaken into the open Thermos. The vial prevents transfer of heat from the hands to the frozen alga. The stopper is replaced quickly and the Thermos is shaken. During the next few minutes the lowest temperature is noted. The measurement is completed by weighing the alga after drying it for two hours in an oven set at 100° C. The amount of water and dry substance in the initially frozen material is then computed.

If no ice is formed the number of calories supplied to the alga is proportional only to its weight and the temperature interval through which it is warmed. In the absence of any change of state the specific heat is nearly constant with temperature. If ice is present the calorimeter must supply 80 additional calories to melt each gram.

The calories supplied to the alga by the calorimeter are equal to the temperature drop observed times the heat capacity which has been determined by calibration with ice. Part of these calories go to warm the dry substance and the water from the cold box temperature T_1 up to the final calorimeter temperature T_2 . This is equal to

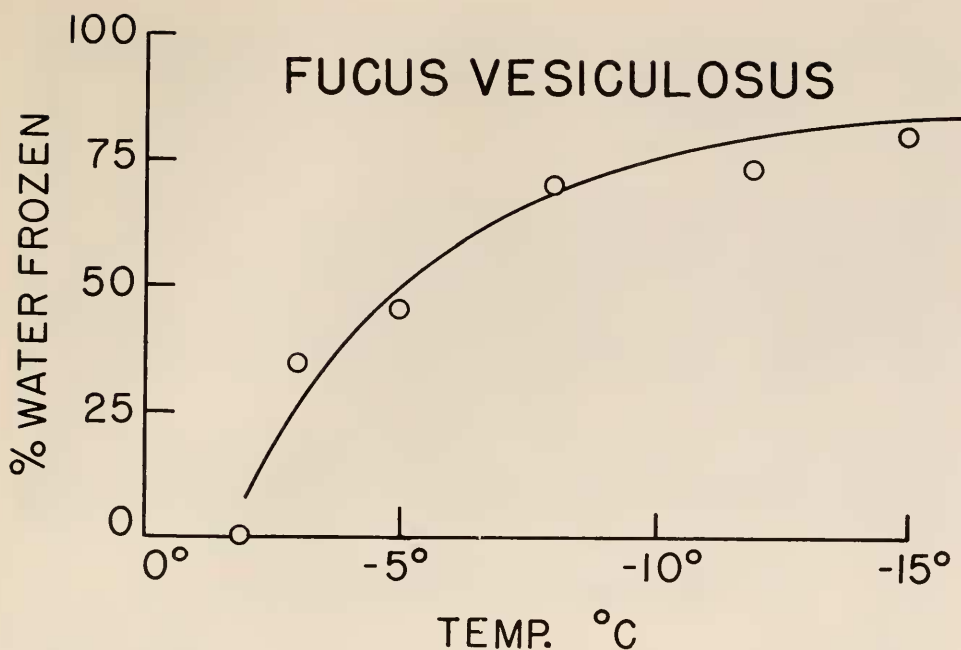
$$(T_2 - T_1) \times (0.3 \times \text{dry wt.} + \text{wt. of water}),$$

where 0.3 has been separately determined to be the specific heat of the dry substance. That of water is 1.0. The remainder of the calories melt any ice that is present. This is converted to grams of ice. Since the water in the starting sample is known, the fraction of it frozen at the temperature T_1 has been determined. A small correction is necessary because the specific heat of ice is only half that of water (Ditman *et al.*, 1942).

2. Results

Figure 1 is typical of the data thus obtained. It is a plot of the per cent of water that is converted to ice at various freezing temperatures in *Fucus vesiculosus*. Similar curves were obtained for *Ascophyllum nodosum*, *Chondrus crispus*, and *Ulva lactuca*. The following table shows the percentage of water as ice at -15° C., the lowest temperature used.

It is evident that a large fraction of algal water is readily frozen at temperatures which frequently occur in nature. The large surface area of *Ulva* would tend

FIGURE 1. Freezing curve of *Fucus*.

to absorb heat during the transfer to the calorimeter. This may account for its having the least ice in Table I.

II. WATER CONTENT IN ALGAE NATURALLY DRIED

Exposed upper intertidal algae become very obviously dried on a windy day when the relative humidity is low. Isaac (1933) measured a 68 per cent loss in weight in *Pelvetia canaliculata* during normal exposure on the short. He did not determine the dry weight. Feldmann (1937) noted that *Bangia fuscopurpurca* can remain out of the water for periods as long as 15 days and still survive. Zaneveld (1937) found only a 30 per cent weight loss in drying conditions. A series of measurements have been made here to determine how much water is normally lost under such conditions.

When the algae looked very dry at low tide on a windy day, samples were taken and weighed immediately. They were then immersed in sea water for several hours and weighed again. Some of them were used to demonstrate photosynthesis by a method described later. Finally, oven drying and weighing gave the necessary

TABLE I

Species	Per cent ice at -15°C .
<i>Fucus vesiculosus</i>	82
<i>Ascophyllum nodosum</i>	76
<i>Chondrus crispus</i>	74
<i>Ulva lactuca</i>	69

TABLE II

Species	Per cent of water lost
<i>Fucus vesiculosus</i>	91
<i>Chondrus crispus</i>	63
<i>Ulva lactuca</i>	77
<i>Enteromorpha linza</i>	84

data to compute the water present when immersed and also in the dried state. Table II gives the maximum dehydration values found.

To measure the rate of loss and reabsorption of water, pieces of *Fucus* were exposed to room air of 22° C. and 40 per cent relative humidity. Such values are not uncommon in nature. They were weighed at intervals and then replaced in sea water and their weight again followed. From Figure 2 it is clear that severe drying can take place during the length of time of tidal exposure. The rate was increased several fold when a breeze was simulated with an electric fan. Less than an hour after re-immersion most of the lost water has been regained. Thin forms such as *Ulva* probably dry even more rapidly. Where the algae grow in many overlapping layers probably only the uppermost are dried very much. The interest here, however, is only in the maximum drying that can be tolerated.

III. RESPIRATION IN FROZEN AND DRIED ALGAE

1. Method

Respiration is awkward to measure at freezing temperatures with conventional methods such as the familiar manometric technique. The volume change resulting from water turning to ice cannot readily be separated from that due to oxygen consumption. The Winkler method of following the disappearance of dissolved

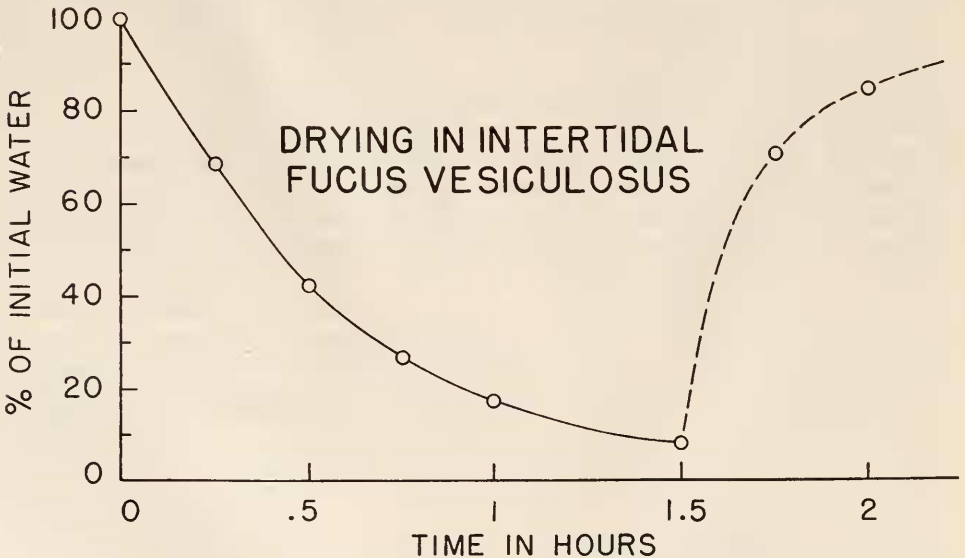


FIGURE 2. Drying curve of *Fucus*.

oxygen cannot, of course, be applied to frozen or dried material. Oxygen consumption in the region of freezing is so sharply temperature-dependent that any method which requires part of the time at higher temperatures is open to gross errors. Scholander *et al.* (1953), in reviewing the literature on respiration in frozen material, concluded that many of the techniques were inadequate for the problem at hand. They used a method of gas analysis which has been applied here with slight modification.

The material is enclosed in a syringe with a known amount of air and kept in the dark. Samples of gas are withdrawn at intervals and analyzed for oxygen. Respiration is computed from the rate of oxygen decrease in the gas phase. Since blanks run in the same way give negligible values, the utilization of oxygen can only be attributed to the frozen or dried algae. The method is specific for oxygen and does not rely on volume decrements which are assumed to result from oxygen being used.

For the low temperature values the algae were placed in darkened hypodermic syringes in a cold bath set at the desired temperature. At least six hours was allowed for the ice-water equilibrium to be reached. The syringes were flushed with chilled outside air and sealed. The tips of the syringes extended above the surface of the coolant. Samples of the gas could be removed without taking the syringes from the bath. The plunger was free to move up as a sample was withdrawn. By thus avoiding any differential pressure when sampling, the danger of leaks is reduced.

The oxygen was measured with the $\frac{1}{2}$ -cc. gas analyzer of Scholander (1947) to 0.02 per cent accuracy. Duplicate analyses were made. When a respiratory period was started by flushing in outside air, the initial concentration was assumed to be 20.94 per cent. Repeated measurements varied between 20.93 and 20.96. Because of the possibility of rate of oxygen consumption depending on tension, oxygen was never depleted below 18 per cent. Two readings were usually taken. The time between these varied from 1 to 200 hours. The slope determined by these points was used to compute the rate of oxygen consumption. The figures are in units of $\text{mm.}^3 \text{ O}_2$ per gram of dry weight of alga per hour.

For the dried algae a fresh sample was weighed fully moist and dried by exposing it to air. It was then weighed again and placed in a syringe. The air in the syringe becomes saturated and no additional water is lost. The readings were made by the same method as for the frozen material. The temperature was kept at 15° C.

Fully moist respiration measurements above 0° were made with volumetric respirometers (Scholander *et al.*, 1952). At 0° these checked with the gas analysis method.

2. Results

Respiration in *Fucus* above and below 0° is plotted in Figure 3. Similar curves were found for *Chondrus* and *Ulva*. The respiration drops sharply below 0°. In the interval from 0 to -10 the apparent Q_{10} is 17. For the same interval above 0° it is 2. The other species had a Q_{10} of 15 and 23 below 0°, respectively, and close to 2 above. At -15° it was necessary to wait 7 to 8 days for the oxygen in the syringe to decrease by a large enough amount to insure an accurate determination.

When the algae were dried, the oxygen consumption again decreased. Figure 4 shows the respiration of *Fucus* related to the degree of dehydration. When 80 per cent of the normal water was lost, the metabolism was down to one-sixth of its normal value. If a sample was allowed to regain water by soaking, the metabolism increased. The solid points were taken consecutively on the same piece of material and showed the reversible nature of the phenomenon. *Chondrus* and *Ulva* showed the same decreased respiration when dried and also recovered the higher rate when re-immersed.

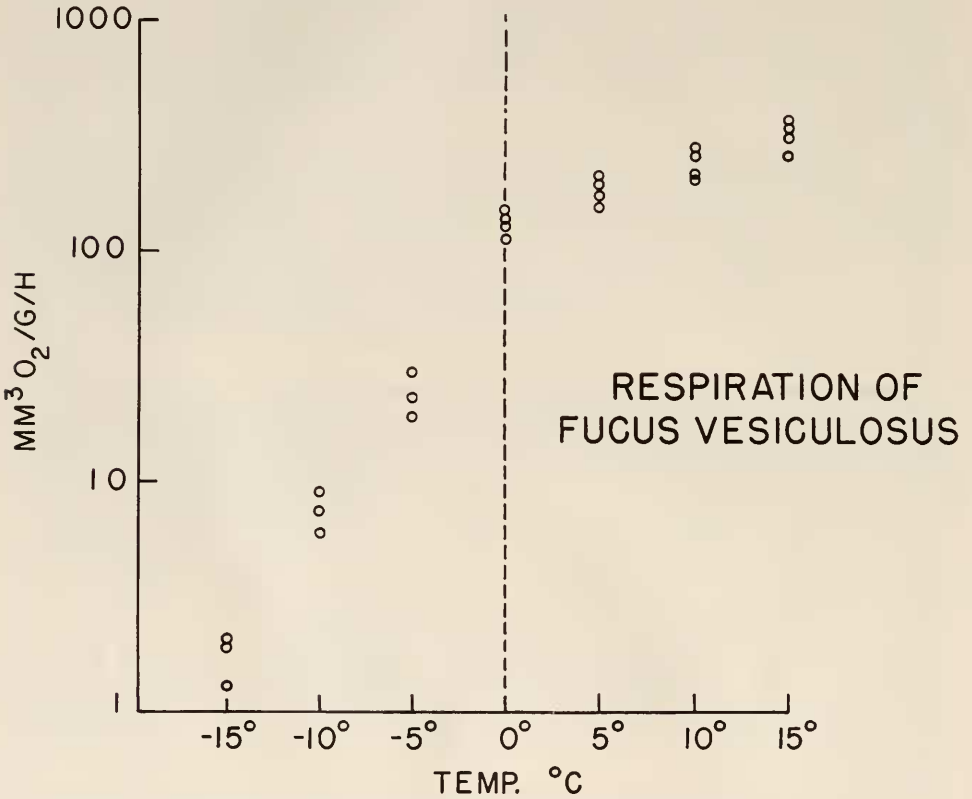


FIGURE 3. Oxygen consumption vs. temperature of *Fucus*.

Loss of water in freezing or drying must increase the salinity of the remaining fluids. Although part of this fluid is in the inter-cellular space, it is in equilibrium with the interior of the cells. Thus loss of any algal water will raise the salinity inside the cells. To determine whether salinity had a specific effect on metabolic rate, higher salinities were made by draining the brine from frozen sea water. Pieces of *Fucus*, *Chondrus*, and *Ulva* were immersed in these for 12 hours. The tissue chloride concentration was measured by acid digestion and titration. It was always proportional to that of the external medium. These species show no evidence of regulating chloride. Respiration rates were measured at the various

salinities. In all cases oxygen consumption decreased less than 30 per cent when the salinity was increased by three times. This concentration is produced at -8°C . by freezing out of water. At such a temperature the respiration is decreased 10- to 15-fold below that at 0° . Salinity is clearly not the primary respiratory depressant when water is lost from the cells by either freezing or drying.

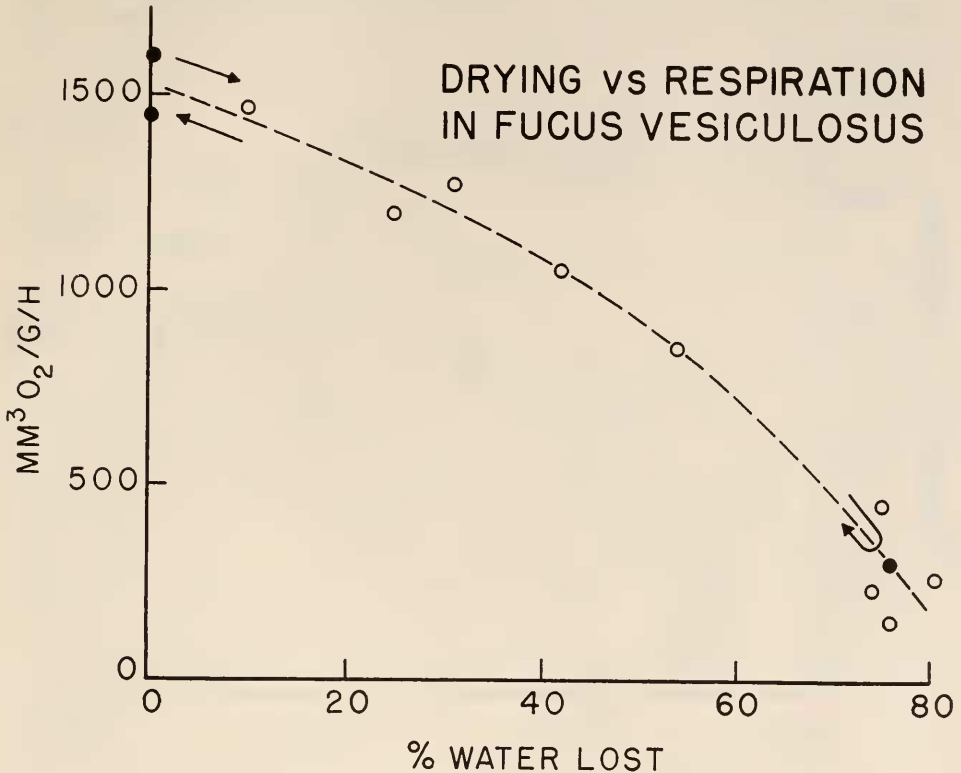


FIGURE 4. Oxygen consumption at various degrees of dehydration.

IV. DISCUSSION

Previous attempts have been made to determine respiratory gas exchange in frozen plants. Scholander *et al.* (1953) measured a precipitous drop in oxygen consumption of several Arctic phanerogams and lichens below 0°C . Ice determinations by a floatation method showed that at -20°C ., more than 90 per cent of the water in some lichens was frozen. They thought it likely the drop in metabolic rate was due to cellular dehydration. They have reviewed the literature and point out that the techniques used previously to measure low temperature respiration were inadequate for the low rates that occur. At freezing temperatures they found a Q_{10} of 20 to 50, while above 0° the same material showed the usual two- to four-times change in oxygen consumption over a ten-degree interval.

Smyth (1934) found a linear relationship between water content and respiration

in lichens. The respiration of air-dried *Acacia* seeds was measured by White (1909) to be only 1/10,000 that of moistened seeds. In dry *Ricinus* seeds he could detect no oxygen consumption. Spores of single-celled forms are dehydrated and are known to show very low oxygen consumption. Respiration in dried algae does not seem to have been previously studied.

The intertidal algae used in these experiments are normally exposed to both freezing and drying. It has been shown here that their respiration under either

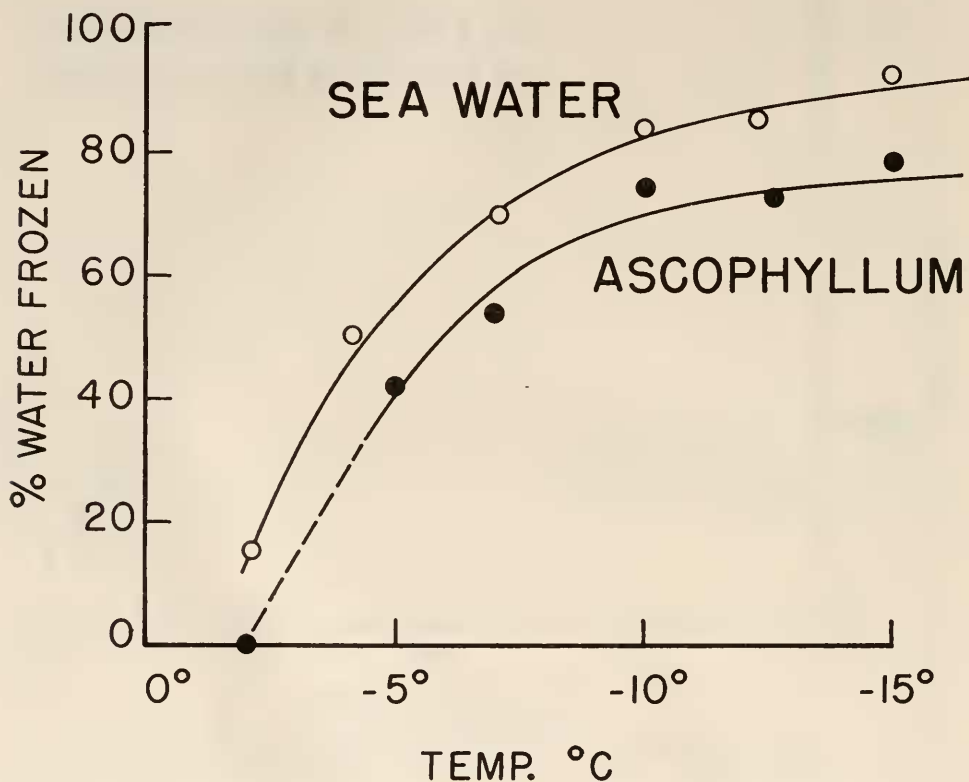


FIGURE 5. Curves showing the freezing of a greater fraction of sea water as compared to algal water.

of these conditions is sharply reduced. In the region of freezing temperatures their Q_{10} is about two times what would be expected from dehydration alone. In going from 0° to -10°, 75 to 80 per cent of the water is frozen. This water loss alone should cause a decrease of six to ten times in oxygen consumption. When the straight temperature effect of a Q_{10} of two is also included, the total apparent Q_{10} should be in the range of 12 to 20. This is in reasonable agreement with those measured directly in the frozen algae.

At progressively lower temperatures calorimetric ice determinations become less accurate. More heat must be supplied to warm the material over the increased temperature range. The calories representing the melting of ice become a smaller

fraction of the total measurement and are thus subject to a larger percentage error. It appears from Figure 1, however, that 15 to 20 per cent of the water is resistant to freezing. The same technique of calorimetry has been applied to a vial of sea water to find the amount of ice at various temperatures. It is compared with *Ascophyllum nodosum* in Figure 5. A larger fraction of the water in sea water freezes at all temperatures than in the algae.

It appears that part of the water in algal cells is unavailable for freezing. Many authors have presented evidence for unfreezable water in gels and also in plant and animal material (Moran, 1926; Greathouse, 1935; review of the literature by Scholander *et al.*, 1953). Moran was unable to freeze all of the water in a gelatin, even at -40° C. The water molecule has a large dipole moment and may well be subject to forces less powerful than conventional bonding but still strong enough to prevent its being frozen. However, Grollman (1931) has rejected the idea of bound water in colloidal systems.

White (1909) found evidence for binding of part of the water in plants at ordinary temperatures. In *Acacia* seeds three per cent remained even after drying over calcium chloride.

Roualt's law expresses a linear relationship between the concentration of a solution and its freezing point depression. If one can assume that the ratio of dry matter to water is equivalent to a concentration, this quantity should increase linearly below freezing. Scholander *et al.* (1953) found this to be so in a *Chironomus* larva. Sea water is nearly linear as would be expected of a solution of crystalloids. The ratio in *Ascophyllum* tends towards a constant value at low temperatures. This could happen if part of the water were bound in such a way that it would not freeze. It would also result from any of the dissolved substances coming out of solution. The concentrations increase as water is frozen out while at the same time the solubility must decrease with temperature. In a frozen alga at -15° C., the solubility of sodium chloride has been exceeded. The cells can either maintain a supersaturation or must be able to actually cope with internal salt crystals.

Siminovitch and Briggs (1949) measured an increased mobility of water in the frost-hardy cells of the black locust, *Robinia*. They thought this was necessary to allow a more rapid exit of water from the cells when intercellular freezing occurs. Such is likely the case with the algae used here when they undergo rapid freezing and drying. It is generally believed that internal freezing is lethal to cells, probably by the physical disruption of ice crystals in the protoplasm. Direct observation will be necessary, however, to determine the locus of this ice.

The lowered respiration observed in the frozen and dried algae may be of value to them in surviving these periods of stress. There can be little growth at such times since the usual supply of nutrients from the sea water is not available. When the alga is frozen, the light available for photosynthesis is usually limited, such as during the Arctic winter. The slowing-down observed here represents a less serious drain on the food stores. The ability of these algae to survive in the intertidal zone may, however, be merely a case of their not being injured by the freezing and drying that are inevitable in such a location.

Respiration has been called the flame of life. In algae at low temperatures it burns very low but is never entirely out.

V. OBSERVATIONS OF ARCTIC FUCUS

The author was a member of an expedition to Hebron in northern Labrador, sponsored by the Arctic Institute of North America in 1954. On arrival early in July, *Fucus* was abundant in the intertidal zone along much of the coast. Large reproducing plants were common although the ice had been gone only a month. It seemed certain they had not grown this much in the brief period of open water. Yet during the winter the ice is several feet thick and is solid to the bottom along the shore. One was led to believe the *Fucus* was frozen solid in the ice during the entire winter.

During this summer visit, pieces of *Fucus* were cooled to -13° C. in a vial immersed in a salt and ice mixture. Calorimetry showed that $\frac{3}{4}$ of the water was frozen at this temperature. About two grams wet weight of recently frozen *Fucus* were put in a 20-cc. syringe with sea water. The syringe was placed in the sun and kept close to 0° by a snow and water mixture. One-ml. samples were removed and analyzed for dissolved oxygen by a gasometric method (Scholander *et al.*, 1955). In 30 minutes the oxygen rose from 3.6 to 8.4 mm³/cc. Soon after this, bubbles formed indicating supersaturation of the dissolved oxygen. Thus the sample of *Fucus* was still able to photosynthesize actively, even directly after being unnaturally frozen during the summer.

Fortunately it was possible to return to the same spot in March and check on the winter condition of the *Fucus*. The ice was many feet thick along the shore. In places tidal stresses had buckled it and the projecting sheets contained some of the algae frozen into the ice when it formed. This *Fucus* was fully exposed to the air temperatures which, during the brief visit, were as low as -26° C. Earlier in the winter they had dropped to -40° C. or lower.

Pieces of ice with *Fucus* in them were chipped free and thawed. The melted water contained only about 0.3 per cent salt. The alga was again checked for photosynthesis with the same positive result. The winter respiration rate was found to be close to that in the summer. There was no sign of a large oxygen debt from the long period in the ice. The *Fucus* is apparently ready to start active growth again in the spring where it left off in the fall.

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SUMMARY

1. As much as 80 per cent of the water in intertidal marine algae is frozen when exposed to the low air temperatures that regularly occur in nature.
2. The same species may lose 90 per cent of their water by ordinary drying during tidal exposure.
3. Metabolism is greatly depressed in both the frozen and dried states.
4. The ability to withstand drying may be related to freezing hardness.
5. Some extreme conditions in the Arctic are described. *Fucus* spends many months frozen into the sea ice at temperatures down to -40° C., yet it is capable of photosynthesis immediately upon being thawed out.

LITERATURE CITED

- BIEBLE, R., 1939. Über die Temperaturresistenz von Meeresalgen verschiedener Klimazonen und verschieden tiefer Standorte. *Jahrb. Wiss. Bot.*, **88**: 389-420.
- DITMAN, L. P., G. B. VOGT AND D. R. SMITH, 1942. The relation of unfreezable water to cold hardness of insects. *J. Economic Entomology*, **35**: 265-272.
- FELDMANN, J., 1937. Recherches sur la végétation marine de la Méditerranée. La côte des Albes. *Rev. Algologique*, **10**: 1-399.
- GORTNER, R. A., 1937. Selected topics in colloid chemistry. Cornell Univ. Press, Ithaca, New York.
- GREATHOUSE, GA. A., 1935. Unfreezable and freezable water equilibrium in plant tissues as influenced by sub-zero temperatures. *Plant Physiol.*, **10**: 781-788.
- GROLLMAN, A., 1931. The vapour pressure of aqueous solutions with special reference to the problem of state of water in biological fluids. *J. Gen. Physiol.*, **14**: 661-683.
- ISAAC, W. E., 1933. Some observations and experiments on the drought resistance of *Pelvetia canaliculata*. *Ann. Bot.*, **47**: 343-348.
- KANWISHER, J. W., 1955. Freezing in intertidal animals. *Biol. Bull.*, **109**: 56-63.
- MORAN, T., 1926. The freezing of gelatin gel. *Proc. Roy. Soc. London, Ser. A*, **112**: 30-46.
- MORAN, T., 1935. Bound water and phase equilibria in protein systems: egg albumin and muscle. *Proc. Roy. Soc. London, Ser. B*, **118**: 548-559.
- SCHOLANDER, P. F., 1947. Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. *J. Biol. Chem.*, **167**: 235-250.
- SCHOLANDER, P. F., C. L. CLAFF, J. R. ANDREWS AND D. F. WALLACH, 1952. Microvolumetric respirometry. *J. Gen. Physiol.*, **35**: 375-395.
- SCHOLANDER, P. F., L. VAN DAM, C. L. CLAFF AND J. W. KANWISHER, 1955. Micro-gasometric determination of dissolved oxygen and nitrogen. *Biol. Bull.*, **109**: 328-334.
- SCHOLANDER, P. F., W. FLAGG, R. J. HOCK AND L. IRVING, 1953. Studies on the physiology of frozen plants and animals in the Arctic. *J. Cell. Comp. Physiol.*, **42**: 1-56.
- SIMINOVITCH, D., AND D. R. BRIGGS, 1949. The chemistry of the living bark of the black locust tree in relation to frost-hardiness. *Arch. Biochem.*, **23**: 8-17.
- SMYTH, E. S., 1934. A contribution to the physiology and ecology of *Peltigera canina* and *P. polydactyla*. *Ann. Bot.*, **48**: 781-818.
- WHITE, J., 1909. The ferments and latent life of resting seeds. *Proc. Roy. Soc. London, Ser. B*, **81**: 417-442.
- ZANEVELD, J. S., 1937. The littoral zonation of some Fucaceae in relation to desiccation. *J. Ecology*, **25**: 431-468.